Characterisation of surfactant-expressing bacteria and their potential bioremediation properties from hydrocarbon-contaminated and uncontaminated soils.

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THIS WORK

- Bacteria produce surfactants with different surface activities and behaviours in air-water and oil-water mixtures and have various biological roles and biotechnological applications.
- The aim of this work was to investigate characteristics associated with oil degradation amongst bacteria isolated from hydrocarbon-contaminated soils in Nigeria and the UK in order to identify isolates with biodegradation properties. The specific focus were to isolate and characterise surfactant-producing bacteria for potential biodegradation among the surfactant-producing strains. Another focus was to investigate via bioinformatics analysis genes responsible for surfactant production and biodegradation of hydrocarbons in the strains.
- Of five sites sampled, a total of 1460 colonies were tested using the drop collapse assay, and 168 were found to express surfactants reducing aqueous liquid surface tensions as assessed by quantitative tensiometry (data not shown) to between 24.7 $mN.m^{-1}$ and 26.7 $mN.m^{-1}$ (Tukey-Kramer HSD, α = 0.05). From these, 6 strains showing significant surface tension reducing abilities and 6 strains expressing no surfactants under the conditions tested were chosen for further analysis from each site.
- Sixty strains were selected and their phenotypes were assessed by a series of growth and behaviour-based assays. Furthermore, their tolerance to varying concentrations of heavy metals and temperature ranges were assessed. Their bioremediation potential was assessed by their ability to utilize diesel as a carbon source and their lipase production potentials were also assessed. The physiochemical properties of the lipase activity were assessed from selected strains.
- Furthermore, bioinformatics analysis of draft whole genome sequences of the eight strains was done to investigate the nature of surfactants, heavy metal resistance mechanisms and hydrocarbon-degrading enzymes expressed by the strains.

FINDINGS

i. Of 168 strains found to express surfactants (24.7 mN/m – 26.7 mN/m, Tukey-Kramer HSD, α = 0.05), 60 strains were selected and when investigated by Hierarchical cluster analysis demonstrated considerable phenotypic diversity (**figure 1**). Eight out of the 60 strains could grow at high temperature (50 °C) while 35 of the 60 strains utilized diesel as a sole carbon source.

ii. Specific lipase activities of eight strains selected from the 60 ranged from 17.12 – 67.42 U/mg (soluble-fraction) and 30.45 – 130.06 U/mg (insoluble-fraction). pH stability profiling revealed optimum lipolytic activity at pH 7 with strain D3 showing highest activity. Furthermore, strain B5 showed a high activity at pH 10, suggesting potential for industrial application. Salt tolerance profiling showed an expected decrease in lipolytic activity with increasing NaCl concentration. Temperature profiling showed optimum lipolytic activity at 30 °C.

iii. Identification by 16S rDNA sequencing revealed that some of the strains belong to *Pseudomonas*, *Bacillus*, and *Stenotrophomonas* genera.

iv. Bioinformatics analysis of the eight draft whole genome sequences via AntiSMASH and RAST revealed the presence of NRPS-like clusters (probable surfactant sequences, **table 1**), cytochrome P450 sequences (five strains), catechol-1,2/2,3-dioxygenase sequences (seven strains), lipase gene sequences (eight strains), and heavy metal resistance gene sequences.

Phenotypic characterisation

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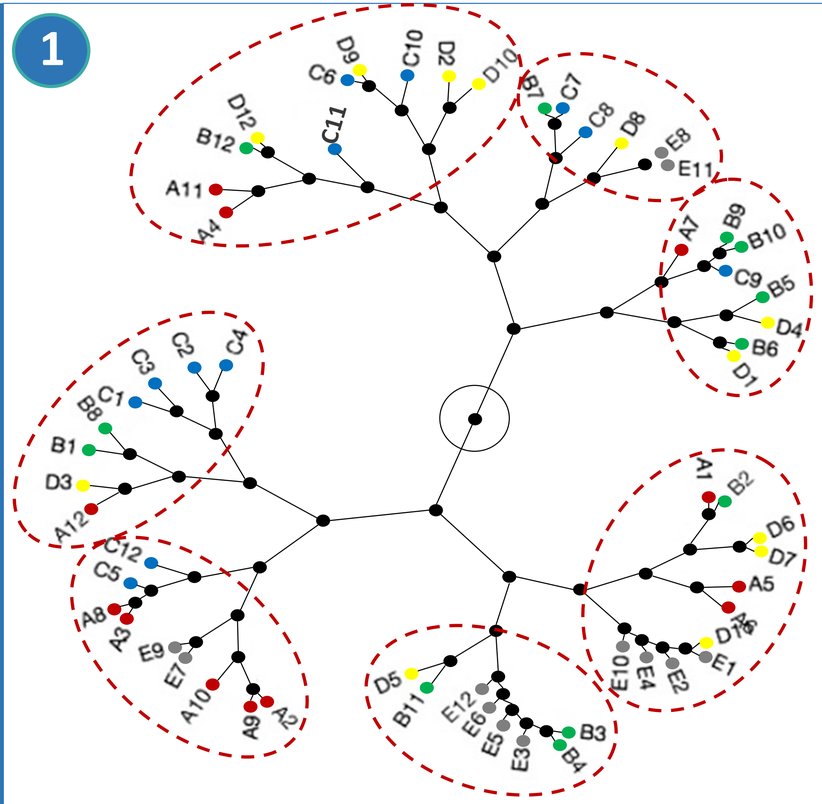

The isolated bacteria can be clustered using simple growth and behavioural assays. Assays including swarming, swimming and twitching motility, expression of catalase, oxidase, protease and lipase, and Gram staining were used to assess the phenotypes of the randomly selected strains. These strains were assessed for similarity using Hierarchical Clustering analysis (HCA) of the phenotypic data. Shown above is the constellation dendrogram in which the randomly selected strains from the four sites (A, B, C and D) showed diversity within the collection.

Table 1: Potential NRPS genes identified in the draft whole genome sequences

Strain	CDS cluster	Name	Size (aa)	First 50 aa for identification	Similar biosynthetic cluster (% similarity)
TA2	04041	srfAC	1276	MSQFSKDQVQDMYYLSPMQEGMLFHAILNPGQSFYLEQITMKVKGSLNIK	Surfactin (100%)
	04042	srfAB	439	MLHAAKGFDPERVEKTLQALIEHHDALRMVYREENGDIVQAYKPIGESKV	
TA7	02073	grsB	4339	MHFSELMAAISTRAIRLQQEDEDLVILGSDDALDDALWDSLAAHKAQLLE	Anikasin (100%)
	02507	lgrB_1	4333	MTDAFELPSTLVQALQRRAALTPDRLALRFLAENEEQAVVLSYRELDERA	Pyoverdine (17%)
LLB2	00556	grsB	4301	MRFSELMAVLSTRAIRLQRDDQDLVVHGDDDALEDALWDALAAHKPALLE	WLIP (100%)
LLB5	03463	lgrD_1	1883	MVPTQWVFLAALPLTPNGKLDRKALPAPDASDSQQQYVAPATELERTLAA	Pyoverdine (11%)
GC5	03204	lgrB_2	4343	MMDAFELPTTLVQALRRRAVQEPERLALRFLAEDDGEGVVLSYRDLDLRA	Pyoverdine (24%)
GC9	05819	lgrB_3	5150	MNAEDSLKLARRFIELPVEKRRVFLETLRGEGIDFSLFPIPAGVSSAERD	Pyoverdine (13%)
MD3	Not found				
MD11	04073	lgrB_4	3651	MSGNMAVRIAKRFVSLPLEQRRQFLAKLREDGKDFSLLPVVESRHDFVSI	Pyoverdine (11%)

CDS – conserved domain sequence
NRPS – non-ribosomal peptide synthetase
WLIP – white line inducing principle

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